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TITLE OF THE INVENTION (280 characters max)

IDENTIFICATION OF GENES INVOLVED IN ANGIOGENESIS, AND DEVELOPMENT OF AN ANGIOGENESIS DIAGNOSTIC CHIP TO IDENTIFY PATIENTS WITH IMPAIRED ANGIOGENESIS

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Respectfully submitted,

By ______
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IDENTIFICATION OF GENES INVOLVED IN ANGIOGENESIS, AND DEVELOPMENT OF AN ANGIOGENESIS DIAGNOSTIC CHIP TO IDENTIFY PATIENTS WITH IMPAIRED ANGIOGENESIS

Background of the Invention

1. Field of the Invention

The invention is directed to the identification and isolation of genetic elements related to angiogenesis and to the creation and use of chips containing isolated genetic elements.

2. Description of the Background

Coronary artery disease and peripheral vascular disease are diseases that are endemic in Western society. In this disease the arteries that supply blood to the heart muscle or to the legs become narrowed by deposits of fatty, fibrotic, or calcified material on the inside of the artery. The build up of these deposits is called atherosclerosis. Atherosclerosis reduces the blood flow to the muscle of the heart or legs, which starves the muscle of oxygen, leading to either/or angina pectoris (chest pain), myocardial infarction (heart attack), and congestive heart failure, as the disease involves arteries supplying the heart, or pain in the leg (claudication) or leg ulcers if the disease involves arteries supplying the leg .

The use of recombinant genes or growth-factors to enhance myocardial collateral blood vessel function may represent a new approach to the treatment of cardiovascular disease. Kornowski, R., et al., "Delivery strategies for therapeutic myocardial angiogenesis", *Circulation* 2000; 101:454-458. Proof of concept has been demonstrated in animal models of myocardial ischemia, and clinical trials are underway. Unger, E.F., et al., "Basic fibroblast growth factor enhances myocardial collateral flow in a canine model", *Am J Physiol* 1994; 266:H1588-1595; Banai, S. et al., "Angiogenic-induced enhancement of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs", *Circulation* 1994; 83-2189; Lazarous, D.F., et al., "Effect of chronic systemic administration of basic fibroblast growth factor on collateral development in the canine heart", *Circulation* 1995; 91:145-153; Lazarous, D.F., et al., "Comparative effects of basic development and the arterial response to injury", *Circulation* 1996; 94:1074-1082; Giordano, F.J., et al., "Intracoronary gene transfer of fibroblast growth factor-5 increases blood flow and contractile function in an ischemic region of the heart", *Nature Med* 1996; 2:534-9.

Despite the promising hope for therapeutic angiogenesis as a new modality to treat patients with coronary artery disease, there is still a huge gap regarding what specific strategy will optimally promote a clinically relevant therapeutic angiogenic response. Moreover, there are no clinical studies yet reported definitively demonstrating that currently tested angiogenesis strategies cause functionally relevant improvement in blood flow to the affected tissue.

Summary of the Invention

The present invention overcomes the problems and disadvantages associated with current strategies and designs and provides kits, compositions and methods for angiotyping" individual patients to predict the likelihood of whether a given individual will develop good vs. poor collaterals naturally.

Several animal studies suggest that factors may exist that interfere with collateral growth--these include diabetes and hypercholesterolemia. There are subgroups of patients with coronary artery disease who have poor collaterals, and others who have excellent collaterals. Impaired collateral development occurring

in response to arterial obstructive disease, or in response to angiogenesis interventions, is determined to a large extent by genetic factors (such as specific genetic polymorphisms), and/or by epigenetic factors (such as DNA methylation patterns) that alter the expression of genes encoding angiogenesis factors. Because of the marked individual variability that exists in the capacity to develop collaterals, and that such individual variability is based in large part on genetic and epigenetic differences among patients, it would be important to diagnosis whether 1) a given patient is likely to develop good vs. poor collaterals naturally, and 2) a given patient is likely to respond to a specific therapeutic angiogenesis strategy. Because of these individual differences, angiogenesis treatment can ultimately be tailored to the individual patient. Therefore, this invention will allow, through DNA expression profiling using DNA chips or similar technology, diagnostic "angiotyping" of individual patients to predict the likelihood of whether a given individual will develop good vs. poor collaterals naturally, or in response to specific angiogenesis therapy.

One embodiment of the invention is directed to methods for "angiotyping" individual patients to predict the likelihood of whether a given individual will develop good vs. poor collaterals naturally. Accordingly, this can involve obtaining and providing a list of genes involved in collateral development.

Another embodiment of the invention is directed to methods for "angiotyping" individual patients to predict the likelihood of whether a given individual will develop good vs. poor collaterals in response to specific angiogenesis therapy.

Another embodiment of the invention is directed to methods for the detection of good vs. poor collaterals, comprising the detection of single nucleotide polymorphisms (SNPs) of an array of genes that have been determined through our experimental studies as being differentially expressed in tissues in which collaterals are developing in response to arterial occlusion. SNPs are detected using microchips or similar technology assaying for all, or most, of the genes determined to play a role in collateral development. The presence of a predisposition to develop poor vs. good collaterals is indicated by the presence of SNPs involving one or more of the genes we have determined are involved in those processes leading to enhanced collateral development.

Another embodiment of the invention is directed to methods for the detection of good vs. poor collaterals, comprises the detection of alterations of proteins in the blood, expressed by the array of genes that have been determined through our experimental studies as being differentially expressed in tissues in which collaterals are developing in response to arterial occlusion. Protein levels will be either higher than normal levels, lower than normal levels, or the proteins will be post-translationally modified, such as, but not limited to changes in phosphorylation states. The determination of such protein levels/modifications can be by standard assays of individual proteins (ELISA, etc), or by newer methods, such as proteomic analysis. The presence of a predisposition to develop poor vs. good collaterals is indicated by the presence of lower or higher blood levels of proteins that are encoded by one or more of the genes we have determined are involved in those processes leading to enhanced collateral development.

Another embodiment of the invention is directed to methods for the detection of good vs. poor collaterals, comprises the detection of DNA

methylation patterns involving those genes that have been determined through our experimental studies as being differentially expressed in tissues in which collaterals are developing in response to arterial occlusion. The presence of a predisposition to develop poor vs good collaterals is indicated by the presence of DNA methylation patterns that alter gene expression, resulting in lower or higher blood levels of proteins that are encoded by one or more of the genes we have determined are involved in those processes leading to enhanced collateral development.

Another embodiment of the invention is directed to kits suitable for performing genetic microarray analysis for detection, where the kit comprises microchips containing the SNPs of most or all of the genes we have determined are involved in those processes leading to enhanced collateral development. The genes may be selected from the group of genes listed in Table 1. The sample may comprise, lymph, venous or arterial blood, and/or vascular tissue of the individual. In one embodiment the polymorphisms are detected using a genetic microarray. In another embodiment the polymorphisms are detected using quantitative PCR.

Another embodiment of the invention is directed to kits for carrying out any of the methods described above.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Other embodiments and advantages of the invention are set forth, in part, in the following description and, in part, may be obvious from this description, or may be learned from the practice of the invention.

Description of the Figures

Table 1 lists the genes whose expression was detectably altered during the development of collaterals.

Description of the Invention

As embodied and broadly described herein, the present invention is directed to kits, compositions and methods for angiotyping individual patients to predict the likelihood of whether a given individual will develop good vs. poor collaterals naturally and, in particular, in response to specific angiogenesis therapy. Those genes that have altered expression levels during the development of collaterals have been identified, and the changes in gene expression have been quantified. The relative changes in gene expression at different time points during the collateral development process have been measured, and these measurements allow additional insight into the progress and development of collaterals. Moreover, by measuring changes in gene expression, the risk of whether a given individual will develop good vs. poor collaterals naturally or in response to specific angiogenesis therapy can be determined.

Because differential expression of genes is involved in collateral development, changes in the degree of expression, or in the length of time during which they are differentially expressed, lead to different degrees of collateral development. In the context of CAD or PVD, the different degrees of collateral development can cause some individuals to have minimal symptoms in

association with atherosclerotic arterial obstructive disease, and other individuals to have severe symptoms. Changes in the degree of gene expression, or in the length of time during which the genes are differentially expressed, are caused by polymorphisms either in the gene or in the regulatory components of the gene. Alternatively, these changes can be caused by "epigenetic alterations," such as, but not limited to changes in DNA methylation patterns. This invention, therefore, identifies those genes in which polymorphisms or altered DNA methylation patterns can convey susceptibility to the development of either poor vs good collateral development.

The identification of genes that are involved in collateral development allows those genes having changed degree or duration of expression, caused in part by polymorphisms of the gene or alterations in DNA methylation patterns, to be used as targets to identify genetic abnormalities conveying altered capacities to develop collaterals. Identification of polymorphisms or alterations in DNA methylation patterns allows prediction of the risk for poor collateral development in patients prior to the performance of angioplasty procedures or the initiation of angiogenesis therapy. This pre-procedure risk prediction will importantly influence how the patient is treated. Some patients deemed to be resistant to the development of collaterals might be offered bypass surgery or angioplasty. Others might forego angiogenesis therapy and be treated aggressively with brachytherapy (intravascular radiation). Accordingly, the present invention provides new and improved methods for "angiotyping" individual patients to predict the likelihood of whether a given individual will develop good vs poor collaterals naturally or in response to specific angiogenesis therapy.

Moreover, identification of the genes that are abnormally expressed by an individual patient because of either a SNP or an altered DNA methylation pattern, provides new methods for ameliorating or treating the disease by therapy targeted to a specific set or subset of those genes with altered expression. Because different polymorphisms and DNA methylation patterns play a role in the development of collaterals in different patients, the invention allows identification of specific abnormalities that may be characteristic to a specific patient. The invention therefore allows for greater specificity of treatment. A regime that may be efficacious in one patient with a specific polymorphism profile may not be effective in a second patient with a different polymorphism profile. Such profiling also allows treatment to be individualized so that unnecessary side effects of a treatment strategy that would not be effective for a specific patient can be avoided.

Specifically, approximately five hundred and seventy five genes are identified whose expression changes during the course of collateral development. Since the differential expression of these genes is involved in collateral development, changes in the degree of expression, or in the length of time during which they are differentially expressed, could lead to altered capacity to develop collaterals.

Changes in the degree of gene expression, or in the length of time during which the genes are differentially expressed, can be caused by polymorphisms in the gene or in the regulatory components of the gene. Such polymorphisms, conveying an increased risk of disease development, have already been identified for several genes associated with several diseases. This invention, therefore, identifies those genes in which polymorphisms can convey susceptibility to poor

vs good collateral development. Similar predictions can derive from altered gene expression caused by altered DNA methylation patterns, which can relate to specific SNPs, or regulate gene expression independently of SNPs. Subsequent reference, therefore, to prediction of good vs poor collateral development, relate to polymorphisms of the genes identified by this invention, or of their regulatory units, or to altered DNA methylation patterns which in turn alter gene expression.

The change in expression of certain of the identified genes is predictive of the capacity to develop poor vs. good collaterals. By identifying 575 genes whose expression changes during collateral development, the inventors recognize that analysis of greater numbers of polymorphisms or DNA methylation patterns of those genes leads to a greater ability to predict the capacity to develop collaterals. In view of the importance that the identified genes may play in collateral development, an ability to manipulate the expression of those genes may be efficacious in the treatment of arterial obstructive disease. Methods to enhance collaterals may include gene therapy to increase the expression of genes down-regulated during collateral development. Treatment may also include methods to decrease the expression of genes up-regulated during collateral development.

Identification of genes involved in collateral development also makes possible an identification of proteins that may effect the development of collaterals. Identification of such proteins makes possible the use of methods to affect their expression or alter their metabolism. Methods to alter the effect of expressed proteins include, but are not limited to, the use of specific antibodies or antibody fragments that bind the identified proteins, specific receptors that bind the identified protein, or other ligands or small molecules that inhibit the identified protein from affecting its physiological target and exerting its metabolic and biologic effects. In addition, those proteins that are down-regulated during the course of collateral development may be supplemented exogenously to ameliorate their decreased synthesis.

Different polymorphisms and DNA methylation patterns may play a role in collateral development in different patients. Accordingly, the present invention makes possible an identification of specific abnormalities that are characteristic of a specific patient ("angiotyping"), which allows for greater specificity of treatment. A regime that may be efficacious in one patient with a specific polymorphism profile may not be effective in a second patient with a different polymorphism profile. Such a profiling also allows treatment to be individualized so that unnecessary side effects of a treatment strategy that would not be effective for a specific patient can be avoided.

<u>Elucidation of Changes in Gene Expression in Collateral Development</u>

The inventors have identified the genes that undergo changes in expression during collateral development. Those genes are listed in Table 1. The inventors have carried out this analysis using nucleic acid array analysis of murine adductor muscles as described in more detail below.

The mouse is a widely accepted model for the human for vascular studies, and results obtained in the mouse are considered highly predictive of results in humans. Accordingly, it is expected that the changes in gene expression in humans during collateral development will be similar to or essentially the same as those observed in the mouse. Exaggerated changes in the degree of expression in these genes, or in the length of time during which the genes are differentially expressed, will predispose to good vs poor collaterals. Such exaggerated changes

are usually caused by polymorphisms in the gene or in the regulatory components of the gene, and therefore the mouse genes identified as being differentially regulated during the angiogenic process will be homologous to the human genes in which such polymorphisms will be found to convey the ability to form good vs. poor collaterals. Moreover, both mouse and human homologues are known for each of the genes described in Table 1, demonstrating further that the results obtained in the mouse studies will be highly predictive of results obtained in humans.

The genes for which SNPs are identified in a give patient, or altered DNA methylation patterns, that are associated with collateral development, also serve as the target for therapeutic interventions—those genes upregulated during the collateral development can be targeted by therapy designed to decrease gene expression or function of the proteins encoded by these genes; those genes down-regulated during collateral development can be targeted by therapy designed to increase gene expression or function of the proteins encoded by these genes.

Changes in gene expression in the mouse ischemic hindlimb during experimentally induced collateral development have been studied, a model commonly accepted as a reasonable animal model simulating collateral development as it occurs in humans. Sample and control mouse hindlimb tissues were obtained, RNA was prepared from the tissues, labeled cRNA generated from it and analyzed using an Affymetrix GeneChip® mouse Genome. Sample and control tissues were compared and those genes that experienced significant changes in gene expression were identified. For the purposes of this study, a two fold increase or decrease in gene expression was deemed significant, although the skilled worker will recognize that under certain circumstances smaller changes in gene expression may also be significant. Corresponding human genes for each of the genes determined to have a significant change in expression were identified.

Although about 575 genes have been shown to have altered expression in collateral development (Table 1), it is possible to reliably predict good vs poor collateral development by analyzing a subset of a few of these genes. In other embodiments, at least five, ten, fifteen, twenty or fifty genes may be studied or, if desired, all or most of the genes listed in Table 1 can be studied. These genes can also be analyzed for polymophisms or altered DNA methylation patterns that alter gene expression. All of the genes can be analyzed initially, but reliable predictions can be made by analyzing a subset of these genes that contains a few members. In other embodiments, at least five, ten, fifteen, twenty or fifty genes may be studied or, if desired, all or most of the genes listed in Table 1 can be studied, for example, using sequencing, short tandem repeat association studies, single nucleotide polymorphism association studies, etc. In each case, however, it generally is more convenient to study gene expression or polymorphisms in a smaller subset of the genes.

By measuring changes in expression of a set of genes (by blood protein analysis), or by identification of polymorphisms or DNA methylation patterns influencing expression of sets of genes, rather than of a single gene, the present invention provides increased statistical confidence that the changes observed are predictive of poor vs. good collateral development, such as by providing reliable risk profiling of an individual. Thus, a change in expression of a single gene, or a single gene polymorphism, may not increase susceptibility to good vs poor collateral development sufficiently to cross the diagnosis threshold. On the other

hand, coordinated changes in expression of multiple specified genes, due the presence of multiple polymorphisms and/or DNA methylation patterns, is much more likely increase the likelihood of poor vs. good collateral development. This is analogous to the situation of an individual have only one risk factor predisposing to atherosclerosis (elevated cholesterol). Risk is increased markedly as the number of risk factors increase (elevated cholesterol plus hypertension, obesity, smoking, diabetes, etc).

Identification of polymorphisms or alterations in DNA methylation patterns allows prediction of the risk for poor collateral development in patients prior to the performance of angioplasty procedures or the initiation of angiogenesis therapy. This pre-procedure risk prediction will importantly influence how the patient is treated. Some patients deemed to be resistant to the development of collaterals might be offered bypass surgery or angioplasty. Others might forego angiogenesis therapy and be treated aggressively with brachytherapy (intravascular radiation). Accordingly, the present invention provides new and improved methods for "angiotyping" individual patients to predict the likelihood of whether a given individual will develop good vs poor collaterals naturally or in response to specific angiogenesis therapy.

<u>Dysregulation of Multiple Genes that Increase Susceptibility to Poor vs Good Collateral Development</u>

Gene polymorphisms and altered DNA methylation patterns that lead to biologically important alterations in the expression of genes that are differentially expressed during collateral development can be measured directly in patient samples. These samples comprise DNA that is most conveniently obtained from peripheral blood. The present inventors used nucleic acid array methods to identify the complete set of genes that exhibit significantly changed expression during the course of the healing response to acute vascular injury. However, other methods for measuring changes in gene expression are well known in the art. For example, levels of proteins can be measured in tissue sample isolates using quantitative immunoassays such as the ELISA. Kits for measuring levels of many proteins using ELISA methods are commercially available from suppliers such as R&D Systems (Minneapolis, MN) and ELISA methods also can be developed using well known techniques. See for example Antibodies: A Laboratory Manual (Harlow and Lane Eds. Cold Spring Harbor Press). Antibodies for use in such ELISA methods either are commercially available or may be prepared using well known methods.

Other methods of quantitative analysis of multiple proteins include, for example, proteomics technologies such as isotope coded affinity tag reagents, MALDI TOF/TOF tandem mass spectrometry, and 2D-gel/mass spectrometry technologies. These technologies are commercially available from, for example, Large Scale Proteomics Inc. (Germantown MD) and Oxford Glycosystems (Oxford UK).

Alternatively, quantitative mRNA amplification methods, such as quantitative RT-PCR, can be used to measure changes in gene expression at the message level. Systems for carrying out these methods also are commercially available, for example the TaqMan system (Roche Molecular System, Alameda, CA) and the Light Cycler system (Roche Diagnostics, Indianapolis, IN). Methods for devising appropriate primers for use in RT-PCR and related methods are well

known in the art. In particular, a number of software packages are commercially available for devising PCR primer sequences.

Nucleic acid arrays offer are a particularly attractive method for studying the expression of multiple genes. In particular, arrays provide a method of simultaneously assaying expression of a large number of genes. Such methods are now well known in the art and commercial systems are available from, for example, Affymetrix (Santa Clara, CA), Incyte (Palo Alto, CA), Research Genetics (Huntsville, AL) and Agilent (Palo Alto, CA). See also US Patent Nos. 5,445,934, 5,700,637, 6,080,585, 6,261,776 which are hereby incorporated by reference in their entirety.

Changes in the degree of gene expression, or in the length of time during which the genes are differentially expressed, can be caused by polymorphisms in the gene or in the regulatory components of the gene. Such polymorphisms, conveying an increased risk of disease development, have already been identified for genes associated with several diseases. The present invention, therefore, identifies those genes in which polymorphisms or altered DNA methylation patterns can convey susceptibility to poor vs good collateral development. It is one object of this invention to identify such polymorphisms by developing a DNA microarray chip containing all those SNPs affecting those genes we have identified as playing a role in collateral development (For example, by using the Affymetrix GeneChip system).

Methods for identifying polymorphisms in genes are well known in the art. See, for example, United States Patent Nos. 6,235,480 and 6,268,146, which are hereby incorporated by reference. Once polymorphisms are identified, methods for detecting specific polymorphisms in a gene using nucleic acid arrays are also well known in the art

Thus, in one embodiment, the invention provides methods where SNPs or altered DNA methylation patterns are identified for at least three genes selected from the genes shown in Table 1. In other embodiments of the invention SNPs or altered DNA methylation patterns are determined of at least five genes to determine the likelihood of good vs poor collateral development. In yet further embodiments the number of genes assayed is ten. In yet other embodiments the number of genes assayed is 20 or at least about 20. In still yet other embodiments the number of genes assayed is 50 or at least about 50. Regardless of the number of genes in the subset of analyzed genes, selected from the genes shown in Table 1, the aggregate number of polymorphisms or DNA methylation patterns can then provide an estimate of good vs poor collateral development. The more biologically significant polymorphisms are present, the greater the risk. As more polymorphisms of the genes listed in Table 1 are identified, even more powerful risk profiling will be possible. Thus, in other embodiments of the invention the expression of at least five genes or at least about five genes is assayed to determine the capacity of collateral development. In yet further embodiments the number of genes assayed is ten. In yet other embodiments the number of genes assayed is 20 or at least about 20. In still yet other embodiments the number of genes assayed is 50 or at least about 50.

The skilled artisan will recognize that, due to the heterogeneous nature of collateral development, not all individuals with poor collateral development will exhibit altered expression of every last one of the genes listed in Table 1. Thus, it is possible that one, a few, or many genes will not exhibit significantly altered

expression (and therefore will contain no biologically important polymorphisms or altered DNA methylation patterns), and that different individuals will exhibit different combinations; yet, the coordinated changes induced by the polymorphisms in the expression of the totality of genes are highly predictive of the presence of prediction of poor vs good collateral development.

In general, where the expression of only a relatively small number of genes is studied, changes in expression in most or all of the genes can be observed to provide a reliable diagnosis of good vs poor collateral development. For example, where only three genes are measured, all three genes can show relevant changes in expression to permit a reliable diagnosis impaired collateral development. Where five genes are studied, changes in at least four genes typically will provide a reliable diagnosis. Where ten genes are measured, a reliable diagnosis is obtained where changes in at least seven genes are observed. Where more than 10 genes are measured, changes in 90%, 80%, 70%, 60% or 50% of the measured genes are predictive of impaired collateral development. As these percentages decrease, the reliability of the diagnosis also decreases, but the skilled worker will recognize that when a coordinated change in expression of 20 or 30 genes of the genes listed in Table 1 is observed this is highly predictive of poor vs good collateral development. In general, as the number of genes increases, it is possible to provide a reliable diagnosis by observing coordinated changes in expression in a relatively smaller subset of the genes studied.

Tissues Sampled to Determine Altered Gene Expression and the Presence of Polymorphisms that Cause Biologically Important Alterations in Relevant Gene Expression

Although any sample containing nucleic acid would be appropriate for this purpose, the simplest tissue to sample is peripheral venous or arterial blood. However, tissue may be used, such as vascular tissue, in particular arterial vascular tissue or venous vascular tissue.

Methods of Studying Gene Polymorphisms, DNA methylation patterns, and protein levels of the Genes Listed in Table 1

Polymorphisms can be identified by several methods including restriction enzyme digestion, sequencing, short tandem repeat association studies, single nucleotide polymorphism association studies, etc. These methods are well-known in the art.

Gene expression can also be studied at the protein level. Gene polymorphisms are detected reliably with tissue derived from any source, including peripheral blood; blood protein levels can serve as a source of identifying altered gene expression.

RNA Expression

Methods of isolating RNA from tissue are well known in the art. See, for example, Sambrook *et al. Molecular Cloning: A Laboratory Manual (Third Edition)* Cold Spring Harbor Press, 2001. Commercial reagents also are available for isolating RNA.

Briefly, for example, cells or tissue are lysed and the lysed cells centrifuged to remove the nuclear pellet. The supernatant is then recovered and the nucleic acid extracted using phenol/chloroform extraction followed by ethanol precipitation. This provides total RNA, which can be quantified by measurement of optical density at 260-280 nM.

[redacted]

mRNA can be isolated from total RNA by exploiting the "PolyA" tail of mRNA by use of several commercially available kits. QIAGEN mRNA Midi kit (Cat. No. 70042); Promega PolyATtract® mRNA Isolation Systems (Cat. No. Z5200). The QIAGEN kit provides a spin column using Oligotex Resin designed for the isolation of poly A mRNA and yields essentially pure mRNA from total RNA within 30 minutes. The Promega system uses a biotinylated oligo dT probe to hybridize to the mRNA poly A tail and requires about 45 minutes to isolate pure mRNA.

mRNA can also be isolated by using the cesium chloride cushion gradient method. Briefly the flash frozen tissue if homogenized in Guanethedium isothiocyanate, layered over a cushion of cesium chloride and ultracentrifuged for 24 hours to obtain the total RNA.

<u>Genetic Microarray Analysis</u>

Microarray technology is an extremely powerful method for assaying the expression of multiple genes in a single sample of mRNA. For example, Gene Chip® technology commercially available from Affymetrix Inc. (Santa Clara, Ca) uses a chip that is that is plated with probes for over thousands of known genes and expressed sequence tags (ESTs). Biotinylated cRNA (linearly amplified RNA) is prepared and hybridized to the probes on the chip. Complementary sequences are then visualized and the intensity of the signal is commensurate with the number of copies of mRNA expressed by the gene.

<u>Protein Expression</u>

Gene expression may also be studied at the protein level. Target tissue is first isolated and then total protein is extracted by well known methods. Quantitative analysis is achieved, for example, using ELISA methods employing a pair of antibodies specific to the target protein.

A subset of the proteins listed in Table 1 are soluble or secreted. In such instances the proteins may be found in the blood, plasma or lymph and an analysis of those proteins may be afforded by any of those methods described for the analysis of proteins in such tissues. This provides a minimally invasive means of obtaining patient samples for estimate of risk of developing restenosis or of atherosclerosis. Methods for identifying secreted proteins are known in the art.

The emerging technology of proteomics can supply a powerful analytic tool to assay for changes in large numbers of proteins.

The following examples are offered to illustrate embodiments of the present invention, but should not be viewed as limiting the scope of the invention.

Examples

Microarray Analysis of the Mouse Hindlimb

<u>Isolation of RNA</u>

Mice underwent femoral artery ligation and extirpation.A control group was treated by sham surgery. Mouse adductor muscles after surgery and sham surgery were collected and flash frozen. Pooled muscles (30-50mg) were crushed into powder using a mortar and pestle (collected with liquid nitrogen) and then homogenized in 2.5 ml of guanidinium isothiocyanate. Total RNA was extracted using ultracentrifugation on cesium chloride cushion gradient for 24 hours at 4°C. See Sambrook et al *supra*.

<u>Target Preparation and DNA Microarray Hybridizations</u>

For the first strand cDNA synthesis reaction, 5.0-8.0 µg of total RNA was incubated at 70°C for 10 minutes with T7-(dT) 24 primer, then placed on ice. For

the temperature adjustment step, 5X first stand cDNA buffer, 0.1 M DTT, and 10 mM dNTP mix was added and the reaction incubated for 1 hour at 42°C. SSII reverse transcriptase was added, and the reaction incubated for 1 hour at 42°C. With the first strand synthesis completed, 5X second strand reaction buffer, 10 mM dATP, dCTP, dGTP, dTTP, DNA Ligase, DNA Polymerase I, and RNaseH were added to the reaction tube. Samples were then incubated at 16 °. Following the addition of 0.5M EDTA, cDNA was cleaned using phase lock gels-phenol/chloroform extraction, followed by ethanol precipitation.

Synthesis of Biotin-Labeled cRNA (*In vitro* transcription)

The synthesis of biotin-labeled cRNA was completed using the ENZO BioArray RNA transcript labeling kit from (ENZO Biochem, Inc., New York, NY) according to the manufacturers protocol. To set up the reaction 1 μg of cDNA, 10X HY reaction buffer, 10X Biotin labeled ribonucleotides, 10X DTT, 10X RNase inhibitor mix and 20X T7 RNA polymerase were incubated at 37°C for 4-5 hours. RNeasy spin columns from QIAGEN were used to purify the labeled RNA, followed by ethanol precipitation and quantification.

Fragmentation of cRNA for Target Preparation

5X fragmentation buffer (200 mM Tris-acetate, pH 8.1, 500 mM KOAc, 150 mM Mg)Ac) was added to the cRNA. Samples were incubated at 94°C for 35 minutes, then placed on ice. Fragmented cRNA was stored at –70°C.

Target Hybridization

Hybridization cocktail was prepared as follows: fragmented cRNA (15 μg adjusted), control oligonucleotide B2 (Affymetrix), 20X eukaryotic hybridization controls (Affymetrix), herring sperm DNA, acetylated BSA, and 2X hybridization buffer (Affymetrix) were combined, and heated to 99°C for five minutes. Hybridization cocktail was then centrifuged at maximum speed for five minutes to remove any insoluble materials from the mixture. Following centrifugation, cocktail was heated at 45°C for five minutes. The clarified hybridization cocktail was then added to the Affymetrix probe array cartridge that had been pre-wet with 1X hybridization buffer. The probe array was then placed in a 45°C rotisserie box oven set at 60 rpm and hybridized for 16 hours.

Washing, Staining and Scanning Probe Arrays

The GeneChip® Fluidics Station 400 was used to wash and stain the array. This instrument was run using GeneChip® software. Briefly, arrays were washed for 10 cycles with non-stringent wash buffer at 25°C, followed by 4 cycles of washing with stringent wash buffer at 50°C. The array was then stained for 10 minutes with Phycoerythrin-streptavidin at 25°C. The array was then washed for 10 cycles with non-stringent wash buffer at 25°C. The probe array was the stained again with phycoerythrin-streptavidin for 10 minutes at 25°C, and then washed for 15 cycles with non-stringent wash buffer at 30°C. Hybridization signals are detected by placing the probe array in an HP Gene Array™ Scanner, which operated using GeneChip® software.

Data Analysis

Data analysis was performed using GeneChip® software (version 3.3) using the manufacturer's instructions. Lockhart, D.J. *et al.*, Nat. Biotechnol. 14:1675-80 (1996). Briefly, each gene was represented and queried by 1-3 probe sets on the chip. Each probe set comprises 16 perfect match (PM) and 16 mismatch (MM) 25 nucleotide base probes. The mismatch has a single base change in the middle of the 25 base pair probe. The hybridization signal from the

PM and the MM probes were compared and this allowed for a measure of signal intensity that is specific and eliminated the nonspecific cross hybridization from the data of the two control chips. Intensity differences as well as ratios of intensity of each probe pair are used to make a "present" or "absent" call. The controls were used as baseline and the experimental GeneChip® assay values compared to the base line to derive four matrixes which were used to determine the difference calls that indicate whether the transcription level of a particular gene is changed.

Iterative comparisons were performed using a spreadsheet analysis (Microsoft Excel). Each experimental data set at a particular time point (n=2) and the difference in expression between the controls and experimental was determined for each gene. Genes with a consistent difference call across all four pairwise comparisons were extracted for further analysis.

<u>GeneSpring® Analysis</u>

The data from each GeneChip® assay was fed into the GeneSpring® software and clustering of genes based on their temporal expression profile was analyzed. Correlation coefficients of 0.97 or greater were taken as a cutoff to create gene-clusters with significant expression homology.

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. All references cited herein, including all U.S. and foreign patents and patent applications, are specifically and entirely hereby incorporated herein by reference. It is intended that the specification and examples be considered exemplary only, with the true scope and spirit of the invention indicated by the following claims.

Claims:

1. A method for the detection of good vs poor collateral development in a mammal, comprising assaying in a sample obtained from said mammal the presence of at least three SNPs or altered DNA methylation patterns of the genes involved in collateral development, as listed in Table 1.

2. The method of claim 1 wherein the presence of good vs poor collateral development is indicated by the presence of at least three SNPs or altered protein or DNA methylation patterns in said sample.

3. The method of claim 1 wherein the presence of good vs poor collateral development is indicated by the presence of at least five SNPs or altered protein or DNA methylation patterns in said sample.

4. The method of claim 1 wherein the presence of good vs poor collateral development is indicated by the presence of at least 10 SNPs or altered protein or DNA methylation patterns.

5. The method of claim 1 wherein the presence of good vs poor collateral development is indicated by the presence of at least 20 SNPs or altered protein or DNA methylation patterns in said sample.

6. The method of any of claims 1-5 wherein said genes are selected from the group consisting of the genes listed in Table 1.

7. The method of claim 1 wherein the assay comprises a genetic microarray.

8. The method of claim 1 wherein the assay comprises quantitative PCR.

9. The method of claim 1 wherein the assay comprises DNA methylation patterns.

10. The method of claims 1-9 wherein the level of gene expression is determined by assaying the level of protein expression in a sample.

11. The method of claim 1 wherein detection is carried out using a kit suitable for performing PCR and wherein said kit comprises primers specific for the amplification of DNA or RNA sequences identified by the genes in Table 1.

12. The method of claim 1 wherein detection is carried out using a gene microarray kit with a chip containing SNPs of the genes depicted in Table 1 and therefore suitable for identifying the presence of SNPs in those genes involved in collateral development and identified in Table 1.

13. A method to estimate the risk of developing good vs poor collateral development comprising detecting the presence of biologically important

polymorphisms, and/or altered protein levels or DNA methylation patterns in at least three genes in a sample obtained from said individual.

14. The method of claim 13 further comprising detecting the presence of biologically important polymorphisms, and/or altered protein levels or DNA methylation patterns in a plurality of genes in a sample obtained from said individual.

15. The method of claim 13 further comprising detecting the presence of biologically important polymorphisms in at least five or ten genes in a sample obtained from said individual.

16. The method of claim 13 further comprising detecting the presence of biologically important polymorphisms, and/or altered protein levels or DNA methylation patterns in at least fifty genes in a sample obtained from said individual.

17. The method of claims 13-16 wherein said polymorphisms, and/or altered protein levels or DNA methylation patterns are selected from the group consisting of the genes listed in Table 1.

18. The method of claim 13 wherein said polymorphisms are detected with a genetic microarray.

19. The method of claim 13 wherein said polymorphisms are detected with quantitative PCR.

20. The method of claims 13-19 wherein detection is carried out with a kit suitable for detecting biologically significant polymorphisms of the genes in Table 1.

Abstract

The invention is directed to methods for "angiotyping" individual patients to predict the likelihood of whether a given individual will develop good vs. poor collaterals naturally. Accordingly, this can involve obtaining and providing a list of genes involved in collateral development. In particular, "angiotyping" individual patients can be used to predict the likelihood of whether a given individual will develop good vs. poor collaterals in response to specific angiogenesis therapy. From an array of genes that have been determined through experimental studies as being differentially expressed in tissues in which collaterals are developing in response to arterial occlusion, single nucleotide polymorphisms (SNPs), or other epigenetic changes, such as DNA methylation patterns, can be identified. SNPs and DNA methylation patterns are detected using microchips or similar technology assaying for all, or most, of the genes determined to play a role in collateral development. The presence of a predisposition to develop poor vs. good collaterals is indicated by the presence of SNPs, and/or alterations in DNA methylation patterns involving one or more of the genes.

TABLE 1

Gene	Genebank #	Product
Fos	V00727	FBJ osteosarcoma oncogene
Timp	V00755	
Rrad	AF084466	Ras-like GTP-binding protein Rad
Scya7	X70058	cytokine
Snk	M96163	
Gp49b	U05265	gp49B2; gp49B1
Tc10l-pending	AW121127	
Krox-24	M28845	zinc finger protein
H3f3b	X13605	H3 histone, family 3B
Emp1	X98471	epithelial membrane protein-1
Alrp	AF041847	cardiac ankyrin repeat protein MCARP
THBS1	M62470	thrombospondin
Scya2	M19681	platelet-derived growth factor-inducible protein
Angptl4	AI326963	
gp49	M65027	cell surface antigen
rrg	D10837	lysyl oxidase
Cdkn1a	AW048937	cyclin-dependent kinase inhibitor 1A (P21)
Litaf-pending	AI852632	
mts1	M36579	
Lgals3	X16834	
Cmkbr5	AV370035	
c-myc	L00039	myelocytomatosis oncogene
Mknk2	Y11092	map kinase interacting kinase
Saa3	X03505	SAA3
Cyr61	M32490	cysteine rich protein 61
pgM	D45889	PG-M core protein
Cish3	U88328	suppressor of cytokine signalling-3
C5aR	S46665	C5a anaphylatoxin receptor
Mt2	K02236	
Zfp36	X14678	zinc finger protein 36
Scya9	U49513	macrophage inflammatory protein-1gamma
Spp1	X13986	secreted phosphoprotein 1
Atf3	U19118	LRG-21
Cd14	X13333	leucine-rich preprotein (AA -15 to 351)
Pde6a	X60664	rod phosphodiesterase alpha subunit
Mmp3	X66402	stromelysin-i

TABLE 1

Lgmn	AJ000990	legumain
C87222	AI836322	
Csf1r	X06368	colony stimulating factor 1 receptor
Cmkbr2	U56819	mcp-1 receptor
Lzm, Lzp, Lys	M21050	lysozyme M
Tdag	U44088	TDAG51
Cyp1b1	X78445	cytochrome P450EF B1
Slfn4	AF099977	schlafen4
E161	X61450	E161
Runx2	AV245229	
Tnc	X56304	precursor tenascin protein
Il17r	U31993	interleukin 17 receptor
S100a10	M16465	calcium binding protein A11 (calgizzarin)
	C85523	
Gro1	J04596	GRO1 oncogene
Pira3	U96684	PIRA3
Itgb2	M31039	complement receptor C3 beta-subunit
Evi2	M34896	ecotropic viral integration site 2
Cish3	AV374868	
Hmox1	X56824	haem oxygenase
Col3a1	AA655199	
Ugdh	AF061017	UDP-glucose dehydrogenase
Tyrobp	AF024637	DAP12
2610024P12Rik	AW124113	
Mt1	V00835	Metallothionein-I
Ywhag	AF058799	14-3-3 protein gamma
Cd68	X68273	macrosialin
Lzp-s	X51547	P lysozyme structural
Fcgr2b	M31312	Fc receptor, IgG, low affinity IIb
Crp2, SmLim	D88792	double LIM protein-1
OTS-8	M73748	glycoprotein 38
TSC-36	M91380	TGF-beta-inducible protein
Mpg-1	L20315	MPS1 protein
Lcn2	X81627	lipocalin
Fkbp10	L07063	FKBP65 binding protein
Col3a1	AV234303	
Anxa1	AV003419	

TABLE 1

Gfpt2	AB016780	Glutamine:fructose-6-phosphate amidotransferase 2
spi2/eb4	M64086	spi2 proteinase inhibitor
Thbd	X14432	thrombomodulin
5730470C09Rik	AA738776	
MRP8	M83218	intracellular calcium-binding protein
2310057H16Rik	AW215736	
Man1a	U04299	mannosyl-oligosaccharide alpha-1,2-mannosidase
Oaz1	AV212241	
Adam19	AA726223	
D15Wsu122e	AW123921	
Mlp	X61399	MARCKS-like protein
Sat	L10244	spermidine/spermine N1-acetyltransferase
Col3a1	X52046	type III collagen
mPHLL2	AB003433	photolyase/blue-light receptor homolog2
	AW047237	
	AI843046	
Angptl4	AA797604	
C1qb	M22531	complement component 1, q subcomponent, beta polypeptide
Apoe	D00466	apolipoprotein
Col14a1	AJ131395	collagen type XIV
Mail-pending	AA614971	
Ftl, Ftl-1	L39879	ferritin L-subunit
Ugt1a6	U16818	UDP glucuronosyltransferase
C1qa	X58861	complement subcomponent C1Q A-chain precursor
Ctss	AJ223208	cathepsin S
1600023E10Rik	AI849082	
2510002C21Rik	AA596710	
Col5a-2	L02918	procollagen type V alpha 2
Scya8	AB023418	monocyte chemoattractant protein-2 (MCP-2) precursor
AI035637	AI842259	
osf-2	D13664	osteoblast specific factor 2 precursor
Eln	U08210	tropoelastin
Stat5b	U21110	mammary gland factor
C1qc	X66295	C1q C chain
Myh8	M12289	
Tubb5	X04663	tubulin, beta 5
PAI-1	M33960	plasminogen activator inhibitor

TABLE 1

metalloelastase	M82831	metalloelastase
Vcl	L18880	vinculin
Sfrp2	U88567	secreted frizzled related protein sFRP-2
Bmk, Hck-1	J03023	hemopoietic cell kinase
Atp1b2	X16645	ATPase, Na+/K+ transporting, beta 2 polypeptide
Slpi	AF002719	secretory leukoprotease inhibitor
Tgif	X89749	mTGIF protein
Gbas	AJ001261	NIPSNAP2 protein
Fgfrp	U04204	aldose reductase-related protein
Anxa4	U72941	annexin IV
Gadd45a	U00937	GADD45 protein
Myf6	X59060	myogenic factor 6 (herculin)
Ext1	X96639	exostoses (multiple) 1
Mrc1	Z11974	macrophage mannose receptor precursor
Il4ra	M27960	interleukin 4 receptor, alpha
Rrm2	M14223	ribonucleotide reductase M2
Npn3	Z31362	
Col5a1	AB009993	collagen a1(V)
Cyba	M31775	
	AF010499	guanidinoacetate methyltransferase
Apbb1ip-pending	AF020313	proline-rich protein 48
Abca1	X75926	ABC transporter
Cmkar4	Z80112	CXCR-4
Cdk7	X74145	protein kinase
2310031E04Rik	AW230891	
Ifnar2	Y09864	soluble type I interferon receptor subunit
Tuba6	M13441	tubulin alpha 6
Fcgr1	M31314	Fc receptor, IgG, high affinity I
Ifi204	M74123	
Pfc	X12905	properdin (AA 5 - 441)
Scyb14	AW120786	
Capg	X54511	Myc basic motif homologue-1
Myo5a	X57377	myosin heavy chain
beta 1	L48687	voltage-dependent Na+ channel beta-1 subunit
Myla	M19436	myosin light chain
2410045D21Rik	AI573601	
Msn	AI839417	

TABLE 1

Sparc	X04017	secreted acidic cysteine rich glycoprotein
1300002F13Rik	AI853531	
8430417G17Rik	AI225296	
Ddah2	AF004106	dimethylarginine dimethylaminohydrolase 2
beta ig-h3	L19932	p68(beta ig-h3)
D5Wsu111e	AA790307	
Gstm3	J03953	
A12	L22977	X-linked lymphocyte-regulated 3b
Cebpb	M61007	alpha-1-acid glycoprotein
	AI841076	
AW549277	AI845902	
flp	M16238	fibrinogen-like protein
1810027D10Rik	AI504305	
Eln	AA919594	
Btg2	M64292	B-cell translocation gene 2, anti-proliferative
Col6a2	Z18272	collagen alpha 2 chain type VI
Peg3	AV353105	
Anxa2	M14044	calpactin I heavy chain
Cebpd	X61800	C /EBP delta
Apod	X82648	apolipoprotein D
Pnp	U35374	purine nucleoside phosphorylase
Ctsl	X06086	cathepsin L
Glk	AV217354	
Il1r2	X59769	type II interleukin-1 receptor
Cd48	X53526	BCM1 antigen
2900055D03Rik	AI839395	
1110032A03Rik	AI851206	
MRP14	M83219	intracellular calcium-binding protein
Fosb	X14897	FBJ osteosarcoma oncogene B
C33, Cd82, KAI1	D14883	C33/R2/IA4
Tnfrsf1b	X87128	p75 TNF receptor
0610011I04Rik	AI787183	
Tubb2	M28739	
Pstpip2	Y18101	macrophage actin-associated-tyrosine-phosphorylated protein
Shc1	AI050321	
THBS2	L07803	thrombospondin 2
Actx	J04181	melanoma X-actin

TABLE 1

Hp	M96827	haptoglobin
Hipk3	AF077660	homeodomain-interacting protein kinase 3
Fxyd5	U72680	ion channel homolog RIC
Bgn	X53928	biglycan (PGI)
Fbn-1	L29454	fibrillin
oxyR	L35599	Y-box binding protein
	AI839289	
Hspa2, HSP70A2	M20567	heat shock protein
Lbp	X99347	LPS-binding protein
C3ar1	U77461	anaphylatoxin C3a receptor
Col1a2	X58251	pro-alpha-2(I) collagen
Cldn5	U82758	lung-specific membrane protein
Pva	X59382	parvalbumin
Lcp2	U20159	SLP-76
Ampd3	D88994	AMP deaminase 3
Col1a1	U03419	alpha-1 type I procollagen
Peg3	AW120874	
Ier3	X67644	
Nfe2l1	AF015881	nuclear factor erythroid-related factor 1
Epcs21-pending	AI853172	
Madh1	U58992	mSmad1
Eif4ebp2	U75530	PHAS-II
Macs	M60474	myristoylated alanine-rich C-kinase substrate
Col6a1	X66405	collagen alpha1 type VI-precursor
	AI019679	
Fn1	M18194	
Krt1-10	V00830	
Grb10	AF022072	adapter protein
	X58196	H19 fetal liver mRNA
C76746	C76746	
Ensa	AJ005985	alpha-endosulfine
helix-loop-helix protein Id2	AF077861	inhibitor of DNA binding 2
Prkar2a	J02935	
Ctsh	U06119	cathepsin H prepropeptide
2510015F01Rik	AW060556	
Txn	X77585	thioredoxin
Bmp1	AA518586	

TABLE 1

Clast1	AB031386	Clast1
Ptx3	X83601	pentaxin related gene
Lxn	D88769	latexin
Cyba	AW046124	
Maged2	AI851574	
2310042E05Rik	AI839731	
Top1	X70956	topoisomerase I
Rnf13	AF037205	RING zinc finger protein
	AA189811	
1300002F13Rik	AW212475	
Sox4	AW124153	
AI413331	AA796989	
JNK2, Prkm9, p54aSAPK	AB005664	JNK2
Tctex1	M25825	t-complex testis expressed 1
Ly111, entactin-2	AB017202	entactin-2
D15Ertd781e	AI528219	
Serpinf1	AF036164	pigment epithelium-derived factor
MS1	L26479	elongation factor-1 alpha
	N28179	
Srst	X67863	simple repeat sequence-containing transcript
Col18a1	L22545	alpha 1(XVIII) collagen
Dnajb9	AW120711	
1200003O06Rik	AI315650	
AW558171	AW120868	
Gus-s	M19279	beta-glucuronidase structural
Snx2	AI842754	
Pftk1	AF033655	Pftaire-1
Ifi30	AI844520	
9130211I03Rik	AA711915	
fisp-12	M70642	FISP-12 protein
Tgfb2	X57413	transforming growth factor-beta2 precurser
Pltp	U28960	plasma phospholipid transfer protein
Cd53	X97227	CD53 antigen
Ncam	X15052	neural cell adhesion molecule NCAM-180
Tnp1	X12521	transition protein 1 (during histone to protamine replacement)
S100a11	U41341	endothelial monocyte-activating polypeptide
Adm	U77630	adrenomedullin precursor

TABLE 1

Tff1	Z21858	pS2m
	AI849721	
Ctsk	AJ006033	cathepsin K
Mapkapk2	X76850	MAP kinase-activated protein kinase 2
Cpo	D16333	coproporphyrinogen oxidase
1600017F22Rik	AV268207	
cyp C	M74227	cyclophilin C
Klkbp	X61597	kallikrein-binding protein
Plod3	AI840146	
3110004L20Rik	AW123347	
edr	AJ007909	erythroid differentiation regulator
2310038G18Rik	AI851313	
	AA002843	
6530405F15Rik	AI644072	
Rbp1	X60367	cellular retinol binding protein I
Nfil3	U83148	NFIL3/E4BP4 transcription factor
AI173274	AI642389	
Gzma	M13226	granzyme A
Myod1	M18779	myogenic differentiation 1
Lama4	U69176	laminin alpha 4 chain
Ig Vheavy-PCG-4	X82692	
Wsb1	AF033186	WSB-1
Tm7sf1	AI060729	
1110004C05Rik	AW125390	
Sap30-pending	AF075136	Sin3-associated protein
AU046135	AI842065	
R75394	AI852838	
Acta1	M12347	alpha-actin
Gltp-pending	AI842825	
Fap	Y10007	fibroblast activation protein
Osmr	AB015978	oncostatin M receptor beta
AW122239	AW122239	
Numb	AV377244	
Dab2	U18869	p67; p96; p93
Actb	M12481	
Atp6n1	U13836	vacuolar adenosine triphosphatase subunit Ac116
1500001M20Rik	AV322862	

TABLE 1

Bgn	AV166064	
Il6st	X62646	gp130
	AI593759	
6330407G11Rik	AV341723	
Gapd	M32599	glyceraldehyde-3-phosphate dehydrogenase
2310010N19Rik	AV335997	
CD106, VCAM-1, Vcam-1	M84487	vascular cell adhesion molecule-1
Capn6	AI747133	
Peg1/MEST	AF017994	Peg1/MEST protein
mptp	M80739	protein tyrosine phosphatase, non-receptor type 2
Evi2	M34896	ecotropic viral integration site 2
Laptm5	AV356071	
sprouty4	AB019280	sprouty-4
Eif1a	AI132207	
5830413E08Rik	AI849939	
Nucb2	AJ222586	precursor NEFA protein
sid478	AB025408	sid478p
Pik3r1	U50413	phosphoinositide 3-kinase p85alpha
Ier2	M59821	growth factor-inducible protein
1300003H02Rik	AW123556	
shrm	AI641895	
Abcc1a	AF022908	multidrug resistance protein
Arhc	X80638	p21RhoC
Mkrn1	AW125438	
hr	Z32675	hairless protein
AI428538	AW048730	
Tieg	AF064088	transcription factor GIF
Col15a1	AF011450	type XV collagen
	AW046449	
Trt	AW122985	
COL9A1L, D6S228E	AB000636	collagen a1 XIX chain
alpha-1 gap junction	M63801	connexin 43
3110003A17Rik	AA833425	
D7Ertd304e	AI157475	
Grb2	U07617	Grb2 adaptor protein
Nramp	L13732	integral membrane protein
TXNRD1	AB027565	thioredoxin reductase 1

TABLE 1

1810003P21Rik	AI844626	
2810417H13Rik	AI122538	
PLA2	M72394	phospholipid-binding protein
Mfap5-pending	AW121179	
Ptprc	M14343	protein tyrosine phosphatase, receptor type, C
Mx1	M21038	Mx1 protein
C80305	AI848825	
Ppicap	X67809	peptidylprolyl isomerase C-associated protein
4922501H04Rik	AI836718	
Ifi204	M31419	interferon-activatable protein
CMH2	L47600	cardiac troponin T
ST2L	D13695	ST2L protein precursor
Acinus-pending	AI839299	
Ifi204	M31419	interferon-activatable protein
Cstb	U59807	cystatin B
	D49733	lamin A
Rpl3	Y00225	J1 protein
Rgs2	U67187	G protein signaling regulator RGS2
Ankrd2	AJ011118	skeletal muscle and cardiac protein
Atp2a1	X67140	mouse fast skeletal muscle SR calcium ATPase
14-3-3 zeta	D83037	14-3-3 zeta
Eif4ebp1	U28656	PHAS-I
Tmsb10	AI852553	
TLR6	AB020808	TLR6
Apobec1	U22262	apolipoprotein B mRNA-editing component 1
2610318G08Rik	AA982595	
Islr	AB024538	ISLR
Bcat2	AF031467	branched-chain amino acid aminotransferase
Krt2-4	X03491	keratin complex 2, basic, gene 4
Mch6, ICE-LAP6, Caspase-9	AB019600	caspase9
Lgl	M34597	immunoglobulin lambda-chain
1110034C02Rik	AI837104	
AI415285	AW049806	
Dlixin, Dlxin1, Dlxin-1,	AB029448	Dlxin-1
Ctsc	U74683	dipeptidyl peptidase I precursor
Mknk2	AI845732	
2810411G23Rik	AI854343	

6043200S.121002

TABLE 1

S100a13	X99921	S100 calcium-binding protein A13
Dscr1	AI846152	
ADFP	M93275	adipose differentiation related protein
Hif1a	Y09085	hypoxia-inducible factor one alpha
Slc16a2	AF045692	X-linked PEST-containing transporter
AA575098	AA575098	
Hif1a	AF003695	hypoxia-inducible factor 1 alpha
EFP, Zfp147	D63902	estrogen-responsive finger protein
Rcal	D13003	reticulocalbin
Ogn	AA647799	
3110046C13Rik	AI172819	
AU043077	AA212964	
AI596360	AI596360	
1810049E02Rik	AA763937	
	X05546	
1110064N10Rik	AW124599	
1110036C17Rik	AW123191	
grg	L12140	amino-terminal enhancer of split
1200007D18Rik	AA815795	
1200012G08Rik	AA880988	
murine CD63	D16432	murine homologue of CD63/ME491
Vps16	AI847040	
4632435C11Rik	AF017639	carboxypeptidase X2
Col6a1	AV010209	
Krt2-16	AV085755	
GTPCH, GTP-CH	L09737	GTP cyclohydrolase I
C77137	C77137	
AA589446	AI849075	
kr, Krml, MafB	L36435	basic domain/leucine zipper transcription factor
Xin	AF051945	Xin
Dnajc3	U28423	p58
Slpi	AV090497	
Surf5	AV264321	
1190002H23Rik	AI854358	
Cma1, Mcp-5, MMCP-5	M68898	chymase 1
Dnajc3	U28423	p58
1110025H08Rik	AV360058	

TABLE 1

0610008L05Rik	AV380793	
D7Wsu105e	AA388099	
	AF073881	myotubularin homologous protein 3
Apaf1	AF064071	apoptotic protease activating factor 1
	AW125241	
P3, DXS253Eh, DXSmhG28	J04761	
Jup	M90365	plakoglobin
p50, WP34, pp52, Lsp-1	D49691	p50b
TMEFF2	AB017270	transmembrane protein with EGF-like and two follistatin-like domains 2
AI853222	AW124544	
AI132321	AW123773	
Adcy7	U12919	adenylyl cyclase type VII
AA407055	AI550305	
	AI837786	
Ednra	AI180687	
Dtx1	U38252	FX-induced thymoma transcript
Aldo1	Y00516	aldolase 1, A isoform
Pros1	L27439	protein S
Diap1	U96963	p140mDia
AI181838	AV316991	
Mmp14	AF022432	matrix metalloproteinase-14
	AI847033	
A1b	U23778	A1-b protein
Usf2	X77602	transcription factor
D730045A05Rik	U69488	viral envelope like protein
C76222	AI846773	
Fosl2	X83971	fos-related antigen-2
Pim1	AA764261	
Midn-pending	AW124785	
1700017B05Rik	AW049360	
Sod3	U38261	extracellular superoxide dismutase
Gnb1	U29055	G protein beta 36 subunit
Psma5	AW048997	
Peg3	AF038939	zinc finger protein
AU021460	AI131895	
Igfbp3	AI842277	
2310021G01Rik	AI606257	

TABLE 1

Akap12	AB020886	SSeCKS
CDK2	AJ223733	cyclin-dependent kinase 2
Ap3s2	U91933	AP-3 complex sigma3B subunit
Uck2-pending	AI850362	
Fbln1	X70853	BM-90/fibulin
Serpinh1	X60676	heat shock protein
Zfp106	AF060245	zinc finger protein 106
MD1, MD-1	AB007599	lymphocyte antigen 86
1200017E04Rik	AW048159	
G6, Clcp	AF109905	Hsc70t; smRNP; G7A; NG23; MutS homolog; CLCP; NG24; NG25; NG26
Ppp4c	AF088911	protein phosphatase X
Arih2	AJ130975	Ariadne-2 protein (ARI2)
Rab7-ps1	Y13361	
3230402M22Rik	AW122364	
Atp6a2	AW123765	
Col6a3	AF064749	type VI collagen alpha 3 subunit
B220, CD45, Cd45, Ly-5, T200, CD45R, Lyt-4	M23158	protein tyrosine phosphatase, receptor type, C
	AA397054	
MSGP-2	D14077	sulfated glycoprotein-2
	AA710439	
AI482343	AW123850	
Cdkn1c	U22399	p57KIP2
C1r	AI132585	
epithelin	D16195	acrogranin precursor
Lipo 1	M69260	lipocortin I
C10	M58004	small inducible cytokine A6
Tnfrsf1a	X57796	55kDa tumor necrosis factor receptor
EGFR	L06864	epidermal growth factor receptor
Lum	AF013262	lumican
Cpt1a	AF017175	carnitine palmitoyltransferase I
Ly6	X04653	lymphocyte antigen 6 complex
Pdk4	AJ001418	pyruvate dehydrogenase kinase-like protein
Slfn2	AF099973	schlafen2
	AB022316	semaphorin W
Col9a3	AW212495	
Gadd45g	AF055638	growth arrest and DNA-damage-inducible 45 gamma
HB-EGF	L07264	heparin-binding EGF-like growth factor precursor

TABLE 1

Lor	U09189	loricrin
tPA, t-PA	J03520	plasminogen activator, tissue
Ppp1r5	U89924	protein phosphatase 1 binding protein PTG
Hsp70-3	M12571	68 kDa heat shock protein
A1d	U23781	A1-d protein
Npn1	Z31360	
Psmd4	AF013099	multiubiquitin-chain-binding protein
Fkbp5	U16959	FKBP51
Ptk9l	Y17808	A6 related protein
Igfbp4	X76066	insulin-like growth factor binding protein 4
Ryr3	X83934	ryanodine receptor type 3
1110027O12Rik	AW212271	
LOC55989	AF053232	SIK similar protein
Mglap	D00613	MGP precursor
4921531N22Rik	AI196645	
	AI841493	
Nfkbia	U57524	I kappa B alpha
Capn3	X92523	calpain
Car2	M25944	
Ces3	AW226939	
Grim19-pending	AI854527	
Cyp2e1	X01026	
adrenodoxin	L29123	iron-sulfur protein
Ckmt2	AV250974	
D16Bwg1543e	AI573367	
Lipe	U69543	hormone-sensitive lipase
Acrp30	U49915	adipoQ
Cycs	X01756	cytochrome c
	AI118905	
myosin light chain 2	M91602	myosin light chain 2
J chain	M90766	joining chain
Aqp4	U88623	aquaporin-4
Retn	AA718169	
Temt	M88694	thioether S-methyltransferase
Mrps7	AI848784	
Igk-V28	M18237	
H2afy	AA646966	

TABLE 1

TIMP-3	U26437	tissue inhibitor of metalloproteinases-3
AW047450	AW047450	
Clcn3	AF029347	chloride channel protein 3
Fmo1	D16215	flavin-containing monooxygenase
2900062L11Rik	AI839718	
	AI852124	
mld, shi, Hmbpr	M11533	myelin basic protein
Cdo1	AI854020	
Amd2	Z23077	S-adenosylmethionine decarboxylase
	AW212131	
Stat1	U06924	Stat1
Rasd1	AF009246	ras-related protein
Aqp4	U48398	mercurial-insensitive water channel 2
MLP, CRP3, MMLP	D88791	muscle LIM protein
Cd1d1	M63695	CD1.1
Mapbpip-pending	AI844560	
Adsl	AA606587	
Akl3l-pending	AI854743	
Fasn	X13135	fatty acid synthase (838 AA)
AA959601	AW125299	
Gstz1	AW060750	
Thrsp	X95279	Spot14
Ldh2	X51905	lactate dehydrogenase 2, B chain
AI848390	AW045204	
Amd2	Z23077	S-adenosylmethionine decarboxylase
Enpp2	AW122933	
Apobec2	AW124988	
Myhcb	AJ223362	slow myosin heavy chain-beta
2310032D16Rik	AW125284	
1110007M04Rik	AA693236	
5730469M10Rik	AI850090	
Gdm1	D50430	glycerol-3-phosphate dehydrogenase
Myh11	D85923	myosin
	AW047232	
0610042C05Rik	AW048828	
	AW047643	
2610100P18Rik	AW123099	

TABLE 1

AAAT, ASCT2	L42115	insulin-activated amino acid transporter
	AA733664	
1110004O20Rik	AW060921	
	AI197161	
AW060987	AI841606	
Pfkfb1	X98848	6-phosphofructo-2-kinase /fructose-2,6-bisphosphatase
Ms4a2	AA797989	
Slc25a15	AA986782	
Iigp-pending	AA914345	
C80633	AI853240	
Tncc	M29793	troponin C, cardiac/slow skeletal
2610042L04Rik	AI853444	
0610011L04Rik	AI849271	
	AI851321	
AA420417	AW123788	
2310061N23Rik	AI158810	
Bet1	AF007552	Bet1p homolog
Gdc1	M25558	glycerolphosphate dehydrogenase 1, cytoplasmic adult
MLC1s, MLC1v	X12972	
Tpm5	U04541	alpha-tropomyosin slow
Mrps25	C77227	